

REMARKS/ARGUMENTS

Reconsideration of this application is requested. Claims 1-11, 21 and 22 are in the case.

I. PRIORITY

The specific reference to the parent application Serial No. 10/433,681 has been completed as suggested on page 2 of the Action. Withdrawal of the objection to the priority claim in this case is now respectfully requested.

II. OATH/DECLARATION

A substitute declaration amended as suggested on page 2 of the Action has been forwarded to the Applicants for execution. The executed declaration will be submitted as soon as received by the undersigned.

III. THE FORMAL 35 USC 112 REJECTIONS

The claims have been objected to and rejected under 35 USC 112 for the reasons detailed on pages 3-6 of the Action. In response, and without conceding to the merit of these rejections, claim 1 has been amended to incorporate changes suggested by the Examiner and other amendments. The changes made are as follows:

Line 3: "comprising" has been replaced by "consisting essentially of";

Lines 4-5: have been modified to provide antecedent for the transcription factor;

Lines 7-9: have been modified to make clear that the inserted sites are substitutions;

Lines 11-13: have been amended to reflect the relationship between the left and right hand side substitutions;

Line 16: has a minor deletion for grammatical purposes;

Line 17: inserts the Examiner's suggested "unmodified" wording;

Lines 19-20: incorporates the necessity for relocation of the packaging signal and limits this to within 600bp of the right hand ITR;

Line 24 includes the Examiner's suggested amendment replacing construct with adenoviral sequence;

Claim 21 has been amended to adopt the changed suggested by the Examiner.

In light of the above, withdrawal of the outstanding objections and rejections under 35 USC 112 is now believed to be in order. Such action is respectfully requested.

V. THE 35 U.S.C. §102 REJECTION

Claims 1, 3, 4, 7, 9-11, 21, 22 and 25 stand rejected under 35 U.S.C. §102(b) as allegedly anticipated by WO 00/56909 to Iggo et al for the reasons of record. This rejection is respectfully traversed.

The claimed invention is in no way suggested by WO 00/56909. That document specifies selected transcription factor binding sites operatively positioned such as to promote expression of ORFs, the products of which are mechanistically directly involved in viral construct nucleic acid replication. E1A ORF product is indirectly involved in such replication. Page 8, lines 3 to 5 list such directly involved products as polymerase, primase, nuclease, helicase, ligase, preferably the DNA polymerase, DNA terminal protein and the DNA binding protein.

Attached is a copy of a web document "Virology Down Under" which evidences that the adenovirus proteins are the DNA polymerase, DNA terminal protein and the DNA binding protein. Moreover, it clear from this document that these are ORFs of E2A and E2B, not E1A.

WO 00/56909 gives one alternative or additional mutation possible as regulation of expression of E1B by mutating that promoter, not E1A. However, page 31, lines 9 to 12 states that such a mutation requires mutation to the E2F promoter to rescue binding protein expression. Thus the E2 promoter taught is not wild type as in the present claim. The option to insert sites in relation to E1A is said to be additional to the E2 mutations (see page 13, lines 12 to 14) of the invention, not alternatives.

The present inventors have now surprisingly determined that maintenance of a strong wild type E2/E3 promoter can be tolerated if E1A, not E1B, is essentially put under control of exogenous transcription factors. This is important as some tumor cell lines are only semi-permissive for the viruses of WO 00/56909 (see present description in PCT as published on page 4, lines 1 to 7). This was not anticipated by WO 00/56909.

In light of the above, it is clear that the outstanding anticipation rejection should now be withdrawn. Such action is respectfully requested.

VI. DOUBLE PATENTING

Claims 23 and 25 have been objected to as allegedly substantially duplicates of claims 2 and 3. In order to reduce the issues in this case, claims 22 and 23 have been

cancelled without prejudice. Withdrawal of this aspect of the double patenting rejection is respectfully requested.

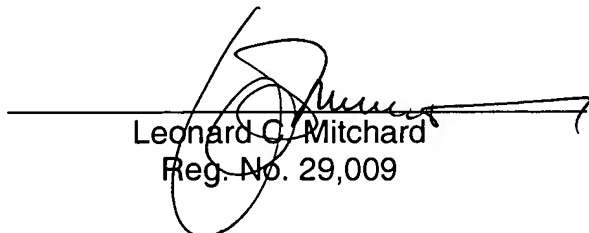
Claims 1-11, 21-23 and 25 stand provisionally rejected on obviousness-type double patenting grounds as allegedly unpatentable over claims 1-4, 7, 9, 11-16, 19, 20 and 25-38 of co-pending application Serial No. 10/433,681. In response, it is requested that this provisional obviousness-type double patenting rejection be placed in abeyance until prosecution has been completed with respect to the applications involved at which time a determination can be made as to whether or not obviousness-type double patenting exists between the various sets of claims. Such action is respectfully requested.

Favorable action is awaited.

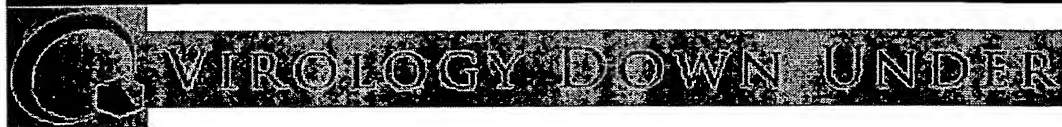
Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____


Leonard C. Mitchard
Reg. No. 29,009

LCM:lfm
901 North Glebe Road, 11th Floor
Arlington, VA 22203-1808
Telephone: (703) 816-4000
Facsimile: (703) 816-4100
Attachment: Web document "Virology Down Under"



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thedisease

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--Latency ??? I hope so.

--Epidemiology: adenoviruses are highly species specific (it's safe to pet your dog). Fecal oral transmission is common in children.

Structure and thefamily

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Basic Properties

Adenoviruses are nonenveloped with an icosahedral structure and a dsDNA genome of 36 kb. The architecture of the virion is unique resembling a WWII mine or a space satellite. Projecting from each of the twelve vertices is the fiber. The protein coat (capsid) is comprised of 252 capsomeres (240 hexons and 12 pentons). penton = penton base + the fiber. Inside the capsid is the viral genome which has the terminal polypeptide (TP) covalently linked to the 5' end at a dCMP residue. Adenoviruses have inverted terminal redundancies comprised of repeats of 100-140bp that vary in number with each serotype. Within the core, polypeptides V and VII are noncovalently linked to the viral DNA. Interestingly, soluble pentons have been shown to be toxic to cells. Adenoviruses are classified within the family Adenoviridae with two genera Mastadenovirus and Aviadenovirus. Human adenoviruses have been classified into six subgroups based on their hemagglutination properties, tumorigenicity, transformation of cells in culture and G+C percentage.

Replicative Cycle

The adenovirus replicative cycle is divided into early and late phases with the late phase occurring when viral DNA replication begins. Normally structural proteins are the major polypeptides synthesized during the late phase. This division into early and late is over-simplified and somewhat misleading as we will see in upcoming revelations. After infection adenoviruses rapidly shutdown host cell macromolecular synthesis i.e. cell DNA and protein synthesis. The mechanism of shutoff is not clear but protein synthesis is rapidly inhibited whereas cell DNA synthesis is shut down at a more leisurely pace. The adenovirus lytic cycle is very efficient producing 10,000 virions per cell with one virus cycle occurring in 32-36 hours.

Attachment-Penetration-Uncoating

Adenovirus attachment is mediated by the fiber which binds a specific receptor on the cell membrane. Subsequently the attached virus migrates to clathrin coated pits to form a receptosome and become internalized. A pH drop in the receptosome alters virion surface properties and results in virion release (with the loss of some viral capsid protein) into the cytoplasm of the cell. The capsid (or what is left of it) migrates to the nucleus via microtubules and viral DNA enters the nucleus (home at last) through nuclear pores. Many if not most virion proteins remain in the cytoplasm (sorry guys). This whole process requires about 2 hours (at 37°C) and will not occur at 0°C. Finally viral DNA is converted into a cell histone complex and may attach to the nuclear matrix for replication.

the genome

Early Transcription

Viral gene expression is not a haphazard process. On the contrary it is highly ordered and carefully orchestrated in much the same manner as genes are regulated for cell division or embryonic development. The ability to function efficiently is critical to the virus's success in a hostile environment where it is dependent on

the host cell for basic macromolecular machinery and supplies. RNA polymerase II is responsible for viral gene transcription at both early and late times. Early RNA synthesis occurs from at least 7 distinct regions of the viral genome, from separate promoters and from both DNA strands. Extensive splicing occurs for both early and late transcripts, an observation that was first documented in adenoviruses. Recent evidence has led to further qualification and subdivision of early transcription. Studies with the protein synthesis inhibitors cycloheximide and anisomycin have shown a defined order of mRNA expression.

- immediate early -- L1
- pre-early -- E1A
- delayed early -- E1B, E2A, E2B, E3, E4
- intermediate -- IVa2, IX
- Rightward transcription.
 - E1A - transformation, viral gene transcription
 - E1B - transformation
 - E3 - nonessential in tissue culture, immune modulation in vivo?
 - L1 - function unknown
- Leftward transcription.
 - E4- immune modulation
 - E2A - DNA binding protein (DBP)
 - E2B - DNA polymerase (140kd) and 80 kd precursor to terminal protein (pTP)

Regulation of early gene expression. The E1A region is pivotal in early gene synthesis. The major product of the E1A is a 13S mRNA which encodes a 289 amino acid protein with diverse functions including the induction of DNA synthesis, induction of mitosis (transformation) and transactivation of viral genes. E1A controls E1B, E2, E3, and E4 mRNA accumulation but cannot itself bind DNA. E1A products appear to activate a series of host proteins which bind to target sequences within early gene promoters.

Early proteins

tumor antigens produced in hamsters bearing adenovirus induced tumors were the first source of information on early proteins. Antisera from these animals reacted with adenovirus infected cells. Since early regions E1A and E1B are the only areas commonly present in tumor cells this led to the early characterization of proteins from these two regions. Subsequent studies employing mRNA hybrid selection and in vitro selection provided more definitive locations for many of the early proteins.

Region E1

his region is essential for adenovirus transformation and both E1A and E1B are required for full transformation. In transformation assays on primary cells in which two oncogenes are required it has been demonstrated that E1A can cooperate with ras and that E1B can cooperate with myc to achieve full oncogenic transformation. Recently it has been demonstrated that E1A protein can bind the tumor suppressor gene Rb105 (Retinoblastoma 105 kd) and that E1B protein can bind the p53 tumor suppressor protein. In highly tumorigenic strains of adenovirus the E1A region down regulates MHC expression. The E1A region produces at least six different polypeptides ranging in size from 38 to 51 kd. The variability in molecular weight is due in part to post-translational modification of the proteins. Two major RNA products are produced by the E1A:

13S and 12S. The 13S mRNA encodes a 289 amino acid protein (51kd) which binds the Rb gene product. The 12S mRNA encodes a 243 amino acid protein (48kd). These two proteins differ only by 46 amino acids which are spliced from the 12S mRNA. The E1B region encodes 3 polypeptides of 19kd, 20kd and 53- 58kd. The 19kd and 53-58kd proteins are important in cell transformation. The 19kd protein transactivates E1A, E1B, E2, E3, E4 and cellular genes such as the heat shock gene. The 55kd (53- 58kd) protein binds the p53 tumor suppressor protein which is also bound by the SV-40 large tumor antigen. Furthermore the 55kd protein interacts with the E4 34kd protein to support efficient synthesis of viral DNA, expression of late genes and host cell shut off.

Region E2

This region is subdivided into two separate transcription regions: E2A and E2B. The E2A region encodes for a single stranded DNA binding protein (DBP) which is heavily phosphorylated at the N terminus. DBP is required for DNA replication and probably also functions in the regulation of transcription. The E2B region encodes for the precursor to the terminal protein (80kd) that is cleaved during viral assembly to 55kd while covalently bound to DNA. This region also encodes the 140kd DNA polymerase.

Region E3

This region is nonessential in tissue culture and can be deleted or replaced without disrupting viral replication. It encodes a 19kd protein that blocks MHC transport to the plasma membrane and a 14.7kd protein that inhibits lysis of adenovirus infected cells by tumor necrosis factor (TNF). In addition, it encodes a 10.4kd protein which binds to the EGF receptor. Thus, the E3 region seems to contain genes which are involved in the modulation of host response to infection.

Region E4

This region encodes a number of polypeptides and at least two of its products have been assigned a function. The 11kd protein binds the nuclear matrix and a 34kd protein binds to the E1B 55kd protein.

DNA Replication

Adenovirus DNA replication has been studied both in vitro and in vivo. In fact, adenovirus was the first DNA virus to be successfully replicated in an in vitro system. At least three viral products and four host cell products have been identified which are essential for viral DNA synthesis. Adenovirus replication occurs in the nucleus and is semiconservative with each strand being elongated continuously without Okazaki intermediates. The viral origin of replication is located in the termini of the viral genome. Two types of replication are thought to occur. In type I, strand elongation occurs from duplex DNA with strand displacement. In type II replication occurs from a single stranded template possibly a panhandle type molecule. Because the template is linear not circular priming of synthesis occurs by covalent attachment of a nucleotide to the terminal protein (protein priming model). Viral proteins required for replication: DNA binding protein (DBP); precursor terminal protein (80kd, pTP) and 140 kd viral DNA polymerase. Cellular proteins required for adenovirus DNA

replication:

1. nuclear factor I (NFI); binds the adenovirus origin of replication and is involved initiation and elongation.
2. nuclear factor II (NFII) is a type 1 topoisomerase
3. nuclear factor III is a DNA binding protein that recognizes the sequence ATGCAAAT.
4. ORP A, binds within the first twelve nucleotides of the viral genome.

Late Transcription

Control of late transcription is very complex and little is known about the molecular mechanisms involved in regulating the switch from early to late gene expression. One of the major features of late transcription is the presence of a tripartite leader for late mRNA. Synthesis of late transcripts begin at 16.45 map units and extend to 99 map units. Extensive splicing of this large transcript then occurs. In general there are 5 classes of late transcripts which have variable 5' ends and coterminal 3' ends. VA RNAs. Virus associated RNAs known as VA RNA I and VA RNA II are small (155 bases) RNAs generated by pol III. They do not encode polypeptides and have the potential to form extensive secondary structure. Suggested functions for these RNAs particularly VA I include: 1. regulation of late mRNA splicing; 2. control of the rate of translation of late polypeptides and 3. inhibition of the effects of interferon by blocking binding of dsRNA to cellular protein kinase and by binding the kinase and blocking eIF2 inactivation.

Late proteins

The synthesis of late proteins is maximal 20 hr post-infection. Most late proteins are structural components of the virion but some early genes are also made late.

Techniques of note:

- Hybrid selection \ *in vitro* translation
- Hybrid arrested translation (HART)
- heteroduplex analysis
- S1 nuclease mapping
- *in vitro* replication
- UV mapping of transcripts

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